

## **I. AMENDMENT**

### **In the claims:**

Please amend the claims as set forth in the following listing. This listing of claims will replace all prior versions, and listings, of claims for the present application:

1. (Currently Amended) A non-mediated enzyme electrode for indicating amperometrically the catalytic activity of an oxidoreductase enzyme in the presence of a fluid containing a substance acted upon by said enzyme and of an electric potential on the electrode, said electrode comprising a base substrate on which is provided:
  - (a) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a resin;
  - (b) a top layer printed or coated on the base layer, said top layer comprising a buffer and being soluble in said fluid; and
  - (c) a catalytically active quantity of said oxidoreductase enzyme in at least one of said base layer and said top layer.
2. (Original) An enzyme electrode according to claim 1, wherein the buffer is selected from a group comprising: phosphate, ADA, MOPS, MES, HEPES, ACA, and ACES, or buffers with a  $pK_a 7.4 \pm 1$ .
3. (Original) An enzyme electrode according to claim 1, wherein the buffer has a pH in the range 7 to 10.
4. (Original) An enzyme electrode according to claim 3, wherein the buffer has a pH in the range 7 to 8.5.
5. (Original) An enzyme electrode according to claim 1, further including a system stabiliser in the top layer, comprising a polyol which is not acted upon by the enzyme.

6. (Original) An enzyme electrode according to claim 5, wherein the system stabiliser is trehalose.
7. (Original) An enzyme electrode according to claim 1, wherein the oxidoreductase enzyme is glucose oxidase.
8. (Original) An enzyme electrode according to claim 1, wherein the base layer also contains particles of finely-divided carbon or graphite.
9. (Original) An enzyme electrode according to claim 8, wherein said finely divided particles of platinum group metal or oxide are adsorbed onto the surface of the finely-divided carbon or graphite.
10. (Original) An enzyme electrode according to claim 8, wherein the particles of finely divided carbon or graphite comprise carbon, and wherein the base layer further includes a blocking agent for blocking active sites of the carbon particles.
11. (Original) An enzyme electrode according to claim 10, wherein the said blocking agent comprises a protein or a polyol.
12. (Original) An enzyme electrode according to claim 11, wherein the blocking agent is bovine serum albumin (BSA) or trehalose.
13. (Original) An enzyme electrode according to claim 1, wherein the said oxidoreductase enzyme is located substantially in the said top layer.
14. (Original) An enzyme electrode according to claim 1, further including a spreading layer for aiding spreading of the said fluid.

15. (Original) An enzyme electrode according to claim 1, wherein the ratio of buffer to enzyme is in the range 30-80 mol/kg.

16. (Original) An enzyme electrode according to claim 15, wherein the ratio of buffer to enzyme is in the range 40-60 mol/kg.

17. (Currently Amended) A non-mediated biosensor for indicating amperometrically the catalytic activity of an oxidoreductase enzyme in the presence of a fluid containing a substance acted upon by said enzyme, the biosensor comprising:

- (a) a base substrate;
- (b) a working electrode and a reference electrode on the base substrate;
- (c) conductive tracks connected to the said electrodes for making electrical connections with a test meter apparatus;

wherein the working electrode includes:

- (d) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a resin;
- (e) a top layer printed or coated on the base layer, said top layer comprising a buffer and being soluble in said fluid; and
- (f) a catalytically active quantity of said oxidoreductase enzyme in at least one of said base layer and said top layer.

18. (Original) A biosensor according to claim 17, wherein the buffer is selected from a group comprising: phosphate, ADA, MOPS, MES, HEPES, ACA, and ACES.

19. (Original) A biosensor according to claim 17, wherein the buffer has a pH in the range 7 to 10.

20. (Original) A biosensor according to claim 19, wherein the buffer has a pH in the range 7 to 8.5.

21. (Original) A biosensor according to claim 17, further including a system stabiliser in the top layer, comprising a polyol which is not acted upon by the enzyme.

22. (Original) A biosensor according to claim 21, wherein the system stabiliser is trehalose.

23 (Original) A biosensor according to claim 17, wherein the ratio of buffer to enzyme is in the range 30-80 mol/kg.

24. (Original) A biosensor according to claim 23, wherein the ratio of buffer to enzyme is in the range 40-60 mol/kg.

25. (Original) A biosensor according to claim 17, wherein the base layer also contains particles of finely-divided carbon or graphite.

26. (Original) A biosensor according to claim 25, wherein said finely divided particles of platinum group metal or oxide are adsorbed onto the surface of the finely-divided carbon or graphite.

27. (Currently Amended) A method of manufacturing a non-mediated biosensor for indicating amperometrically the catalytic activity of an oxidoreductase enzyme in the presence of a fluid containing a substance acted upon by said enzyme, the method comprising the steps of:

- (a) taking a base substrate having a working electrode and a reference electrode thereon, and conductive tracks connected to the said working and reference electrodes for making electrical connections with a test meter apparatus;
- (b) printing on the said working electrode an ink containing finely divided platinum group metal or oxide and a resin binder;
- (c) causing or permitting the said printed ink to dry to form an electrically conductive base layer comprising the said platinum group metal or oxide bonded together by the resin; and
- (d) forming a top layer on the base layer by printing or coating the base layer with a coating medium comprising or containing a buffer, the top layer being soluble in said fluid; wherein

(e) a catalytically active quantity of said oxidoreductase enzyme is provided in at least one of said printed ink and said coating medium.

28. (Original) A method according to claim 27, wherein the coating medium is a coating fluid containing the buffer and wherein the method further comprises causing or permitting said coating fluid to dry to form a top layer on the base layer.

29. (Original) A method according to claim 28, wherein the coating fluid is applied by drop coating.

30. (Original) A method according to claim 28, further including the step of applying a spreading layer on the base layer prior to application of the coating fluid.

31. (Original) A method according to claim 30, wherein the step of applying a spreading layer comprises applying a surfactant-coated polyester mesh on the base layer.

32. (Original) A method according to claim 30, further comprising the step of applying a first dielectric layer prior to applying the spreading layer, the first dielectric layer being applied around the reference electrode and the working electrode to define a target area to which the said fluid containing a substance acted upon by the enzyme will be applied.

33. (Original) A method according to claim 32, further comprising the step of applying a second dielectric layer around the target area so as to secure the spreading layer in place.

34. (Original) A method according to 28, wherein said enzyme is provided in the coating fluid.

35. (Original) A method according to claim 28, wherein the concentration of buffer in the coating fluid is in the range 300 mmol/L to 1 mol/L.

36. (Original) A method according to claim 27, wherein the ratio of buffer to enzyme is in the range 30-80 mol/kg.
37. (Original) A method according to claim 36, wherein the ratio of buffer to enzyme is in the range 40-60 mol/kg.
38. (Original) A method according to claim 27, wherein the buffer comprises phosphate or ADA.
39. (Original) A method according to claim 27, wherein said finely divided platinum group metal or oxide in said ink is adsorbed on the surface of particles of finely divided carbon or graphite.
40. (Original) A method according to claim 28, wherein the coating fluid has a pH in the range 7 to 8.5.
41. (Currently Amended) A non-mediated enzyme electrode for indicating amperometrically the catalytic activity of glucose oxidase in the presence of glucose in whole blood and of an electric potential on the electrode, said electrode comprising a base substrate on which is provided:
- (a) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a bonding agent;
  - (b) a top layer printed or coated on the base layer, said top layer comprising a buffer having a range from about pH 7 to about pH 8.5, and said top layer being soluble in whole blood; and
  - (c) a catalytically active quantity of glucose oxidase in at least one of said base layer and said top layer.
42. (Currently Amended) A biosensor for indicating amperometrically the catalytic activity of glucose oxidase in the presence of glucose in whole blood, the biosensor comprising:
- (a) a base substrate;
  - (b) a working electrode and a reference electrode on the base substrate;

- (c) conductive tracks connected to the said electrodes for making electrical connections with a test meter apparatus;

wherein the working electrode comprises:

- (d) an electrically conductive base layer comprising finely divided platinum group metal or oxide bonded together by a bonding agent;
- (e) a top layer printed or coated on the base layer, said top layer comprising a buffer having a range from about pH 7 to about pH 8.5, and said top layer being soluble in said whole blood; and
- (f) a catalytically active quantity of said glucose oxidase in at least one of said base layer and said top layer.

43. (Currently Amended) A method of manufacturing a non-mediated biosensor for indicating amperometrically the catalytic activity of glucose oxidase in the presence of glucose in whole blood, the method comprising the steps of:

- (a) taking a base substrate having a working electrode and a reference electrode thereon, and conductive tracks connected to the said working and reference electrodes for making electrical connections with a test meter apparatus;
- (b) printing on the said working electrode an ink containing finely divided platinum group metal or oxide and a bonding agent;
- (c) causing or permitting the said printed ink to dry to form an electrically conductive base layer comprising the said platinum group metal or oxide bonded together by the resin;
- (d) forming a top layer on the base layer by printing or coating the base layer with a coating fluid containing a buffer and having a pH in the range about 7.0 to 8.5, said top layer being soluble in said whole blood; wherein
- (e) a catalytically active quantity of said glucose oxidase is provided in at least one of said printed ink and said coating fluid.